REMARKS/ARGUMENTS

Claims 1-14 and 21-24 are active. Claims 15-18 and 20 were withdrawn from consideration and are cancelled without prejudice to their presentation in a Divisional Application. Minor edits have been made to claims 4 and 14. Claims 21-24 tracks the limitations in claim 1. Specific support for claims 23 and 24 is found on pages 9-10. No new matter is believed to have been added. Favorable consideration of this Amendment and allowance of this application are now respectfully requested.

Restriction/Election

The Applicants previously elected with traverse **Group I** claims 1-4, directed to producing a heterologous RNA of interest. The requirement has been made FINAL. The Applicants respectfully request that the claims of the nonelected group(s), or which are directed to other withdrawn subject matter, which depend from or otherwise include all the limitations of an allowed elected claim, be rejoined upon an indication of allowability for the elected claim, see MPEP 821.04.

Objection

The specification was objected to on the ground that claim 4 contains the term "mitochondrial targeting signal". This issue is moot in view of the replacement of "signal" with "sequence" as described on page 11, line 37 of the specification. Accordingly, this objection may now be withdrawn.

Objection

Claim 4 was objected to as containing at typographical error. This error has been corrected rendering this objection moot.

Rejection—35 U.S.C. §112, second paragraph

Claim 14 was rejected under 35 U.S.C. 112, second paragraph, as being indefinite. This rejection is moot in view of the amendment above.

Aspects of the Invention

The invention provides a method for producing in quantity and at low cost large, pure amounts of an RNA of interest without cellular or mitochondrial RNA contaminants, see page 3, lines 17 to 21 of the specification). This method may be used on an industrial-scale for making any heterologous RNA of interest (i.e., RNA not encoded by the yeast mitochondrial genome, page 5, lines 28-30 of the specification). The inventors have found that mitochondrial transformants of a yeast strain lacking mitochondrial DNA (e.g., *rho*^{0} strain) can produce the RNA of interest economically and free of substantial amounts of other RNA contaminants. Thus, the invention allows the production of said RNA of interest in large amounts, at a low cost, and in a form which is stable and can be readily isolated insofar as the only RNAs produced in the mitochondria of the synthetic *rho*$^{-}$ yeast strain are those which are encoded by the DNA used for the transformation--page 4, lines 20-25 and page 7, line 31 to page 8, line 17 of the specification, in the Example on page 26, line 28 to page 27, line 4, and in Figure 4).

Rejection—35 U.S.C. §103(a)

Claims 1, 2, 5, 7, 8, 11 and 12 were rejected under 35 U.S.C. §103(a) as being unpatentable over Bonnefoy, et al., Meth. Enzymol. 350: 97, in view of Lisowsky, et al., Eur. J. Biochem. 164:559. The cited prior art does not disclose all the elements of the invention,

suggest the combination of elements of the invention, or provide a reasonable expectation of success for producing a heterologous RNA of interest using this method.

Bonnefoy generally discloses genetic transformation of mitochondria in the yeast *Saccharomyces*. As mentioned in the OA, Bonnefoy does not disclose or suggest a process of producing a heterologous RNA of interest; moreover, it does not suggest a method of expressing heterologous RNA in mitochondria lacking mitochondrial DNA. Bonnefoy concerns the genetic transformation of *S. cerevisiae* mitochondria and pertains to how to deliver exogenous DNA into mitochondria and to create directed mutations or insert new genes into mitochondrial DNA (mtDNA) via homologous recombination (see introduction, page 97).

Lisowsky is a research paper describing RNA synthesis in isolated mitochondria (p. 562, bottom of col. 1) and was relied upon as teaching "transforming yeast mitochondria with a plasmid comprising a gene which is expressed to produce RNA" (OA, bottom of page 5). However, Lisowsky, like Bonnefoy does not teach "(1) transforming the mitochondria of yeast cells lacking mitochondrial DNA" as required by the present claims, see claim 1. Thus, these documents in combination do not disclose all the elements of the invention and cannot render it obvious.

Moreover, there cannot be any suggestion to combine the teachings of these two references to arrive at the invention because of the missing elements. However, assuming, *arguendo,* that these documents disclosed each element of the invention, they provided no reasonable expectation of success for a method of producing quantities of a heterologous RNA of interest free of other RNA contaminants . The mitochondria in the Lisowsky yeast contain mitochondrial DNA which would express endogenous mitochondrial RNA. Such a method would not have been effective in recovering only heterologous RNA. Even, if the ordinarily skilled artisan had combined the teaching of Bonnefoy with the teaching of

Lisowsky for making RNA, the ordinary artisan would have made mitochondrial recombinant of yeast expressing the exogenous DNA, grown the recombinants, isolated the mitochondria and extracted the RNA from the isolated mitochondria. This would have resulted in obtaining a mixture of endogenous yeast mitochondrial RNAs and the heterologous RNA of interest.

Nothing in Bonnefoy or Lisowsky suggests using yeast mitochondria devoid of mitochondria DNA to produce a heterologous RNA of interest ("heterologous RNA" is not encoded by the yeast mitochondrial genome, see the specification, page 5, and lines 28-30). Lisowsky teach only the analysis of mitochondrial transcription and do not give any indication that mitochondria could be used to produce a heterologous RNA.

Accordingly, this rejection cannot be sustained because the cited art does not disclose all the elements of the invention, or suggest or provide a reasonable expectation of success for the present invention.

Additional analysis of the cited art and the Examiner's arguments is provided below. The Examiner considers that:

> . . .it would have been obvious to one of ordinary skill in the art to use mitochondria to produce RNA because studying RNA production will allow for a better understanding of how mitochondrial transcription works and Lisowsky et al. teach that the transcription machinery has been difficult to characterize.

As discussed above, there is no motivation in the prior art to transform a yeast strain that does not contain mitochondrial DNA in its mitochondria. Moreover, the teachings of Lisowsky, when read in context fail to suggest or even teach away from the invention. A reference should always be considered for everything it would have fairly taught a person having ordinary skill in the art. *In re Lamberti*, 545 F.2d 747, 750 (CCPA 1976). There is no specific motivation for the combination of the invention in Lisowsky. However, assuming for the sake of argument that the general motivation to experiment with mitochondrial

transcription would have motivated the ordinary artisan to make the invention Lisowsky teaches that the different factors involved in the expression of this genome had not been elucidated (see page 559, first column, beginning of first paragraph). Further, the Examiner points out the lack of a reasonable expectation of success for the invention: "*Lisowsky et al. teach that the transcription machinery has been difficult to characterize*". The Examiner argues that an ordinary artisan would have been motivated to produce the heterologous RNA of made by the invention based on the concluding remarks on page 11 of Bonnefoy: "*manipulation of the S. cerevisiae mitochondrial genome should provide a useful model for* ***other systems*** (emphasis added)". However, when read in context the next sentence page 111 indicates that "*other systems*" do not refer to RNA production but to the experimental analysis of the mitochondrial genome of other single-celled eukaryotes like *Chlamydomonas*. Therefore, nothing in Bonnefoy *et al.* or Lisowsky *et al.* would have motivated the artisan of ordinary skill to use yeast mitochondria that do not contain mDNA to produce a heterologous RNA of interest (i.e., one not encoded by the yeast mitochondrial genome).

The Examiner argues that (end of page 5 to beginning of page 6 of the OA that:

> *Lisowsky et al. teach transforming yeast mitochondria with a plasmid comprising a gene which is expressed to produce RNA, the DNA encoding the RNA being under control of a promoter and terminator that are functional in yeast mitochondria, since the RNA was successfully produced in yeast mitochondria.*

The Applicants respectfully point out that this is not the case. Lisowsky is directed to the analysis of yeast mitochondrial transcription. The purpose of Lisowsky is to identify the proteins of the mitochondrial transcription apparatus (first paragraph of introduction page 559). Lisowsky discloses a method for analysing yeast mitochondrial transcription comprising: (a) culturing yeast cells, (b) isolating the mitochondria from the yeast cells, and (c) extracting the RNA from the isolated mitochondria (see material and methods, page 559).

This method for analysing yeast mitochondrial transcription uses **wild type** and **nuclear temperature-sensitive petite mutant** of *S. cerevisiae*, that are yeast strains having an **intact mitochondrial genome** (rho+, mit+ yeast strains). The teachings of Lisowsky regarding RNA synthesis (transcription of yeast mitochondrial genes) occurring *in vivo* and *in vitro* in yeast mitochondria having a mitochondrial genome (rho+, mit+ yeast strains) and preparation of mitochondrial RNAs (total mitochondrial yeast RNAs) from isolated mitochondria does not suggest or provide a reasonable expectation of success for obtaining heterologous RNA free of RNA expressed by mitochondrial genomic DNA. Moreover, Lisowsky does not teach the transcription of RNA from a heterologous gene of interest (RNA not encoded by the yeast mitochondrial genome) in yeast mitochondria; does not teach the transformation of yeast mitochondria; does not teach a mitochondrial transcription vector; and does teach yeast strain lacking mitochondrial DNA (rho^0 yeast strain).

The Examiner considers that Bonnefoy teaches a mitochondrial transcription vector. This is not true for the following reasons. It is well known in the art that *in vivo* transcription vectors (plasmids, for example) comprise an isolated promoter and an isolated transcription terminator that are recognized by an RNA polymerase and by transcription factors that are present in the cells that are transfected/transformed with said vector. The DNA encoding the RNA of interest is inserted between the promoter and the transcription terminator. Usually, the transcription vector comprises a marker gene which allows the selection of the transformants.

Bonnefoy does not teach a mitochondrial transcription vector, rather they teach transformation vectors that allow the introduction of DNA fragments into the mitochondrial genome by homologous recombination. The DNA that is transformed in the cells is expressed as protein after it has been integrated into the mitochondrial genome. In addition, expression refers to the transcription (RNA synthesis) and the translation (protein synthesis) of the gene

of interest whereas transcription vectors characteristically lack crucial sequences that code for polyadenylation sequences and translation termination sequences in translated mRNAs, making expression (protein synthesis) of transcription vectors impossible. Therefore, expression vectors which allow the transcription and the translation of the gene of interest differ from transcription vectors designed to transcribe RNA, but unconcerned with its expression as protein.

In addition, Bonnefoy teaches that the transformation of *rho*^{0} yeast strains (lacking mtDNA) by microprojectile bombardment is 20 times more efficient than the transformation of rho+ yeast strains (beginning of third paragraph page 109).

Bonnefoy teaches also that *rho*^{0} yeast strains allow DNA introduced from outside the cell (bacterial plasmid DNA) to be propagated within the mitochondria as a plasmid (synthetic rho^{-} molecule) and the plasmid-borne mitochondrial sequences to recombine homologously with complete *rho*+ mtDNA (beginning and end of p. 100). However, Bonnefoy *et al.* do not teach the use of rho^{0} yeast strains for producing RNA.

Furthermore, Lisowsky teach that isolating mitochondrial RNAs synthesized *in vivo* from yeast poses several major problems: RNA degradation (see page 562, first column, beginning of last paragraph and second column, beginning of first and second paragraph) and contamination of RNA preparations with cytosolic 28S and 18S rRNA (Figures 1 and 2 and page 562, first column, beginning of first paragraph). These problems, would have taught the skilled artisan away from using yeast mitochondria for making RNA.

Therefore, since the prior art does not disclose all the elements of the invention—expression of heterologous RNA in yeast mitochondria not containing mitochondrial DNA--, suggest this combination, or provide a reasonable expectation of success for expression of isolated or pure heterologous RNA, this rejection cannot be sustained.

Rejection—35 U.S.C. §103(a)

Claim 3 was rejected under 35 U.S.C. §103(a) as being unpatentable over Bonnefoy, et al., Meth. Enzymol. 350: 97, in view of Lisowsky, et al., Eur. J. Biochem. 164:559, and further in view of Dziembowski, et al., J.B.C. 278:1603. This rejection is not sustainable over the combination of Bonnefoy and Lisowsky for the reasons discussed above. Dziembowski was cited as disclosing strains ΔSUV3 and ΔDSS1. However, Dziembowski does not suggest the elements missing from the two primary references, such as expression of a heterologous RNA in yeast mitochondria lacking mitochondrial DNA. Page 7 of the OA indicates that Dziembowski "state that inactivation of SUV3 or DSS1 results in respiratory incompetence and eventual loss of the mitochondrial genome". However, there is no suggestion to select "yeast cells lacking mitochondrial DNA" to express heterologous DNA, nor does this reference provide a reasonable expectation of success that such expression would be useful or even that it would take place. Accordingly, this rejection cannot be sustained.

Rejection—35 U.S.C. §103(a)

Claim 4 was rejected under 35 U.S.C. §103(a) as being unpatentable over Bonnefoy, et al., Meth. Enzymol. 350: 97, in view of Lisowsky, et al., Eur. J. Biochem. 164:559, and further in view of Komiya, et al., J.B.C. 269:30893 and Hwang, et al., J. Virol. 74:4074. This rejection cannot be sustained for the reasons discussed above. Komiya and Hwang were relied upon, respectively, as disclosing "viral RNAP integrated into the genome of *Pichia*" and "using a mitochondrial targeting signal for cytosolic import". However, these secondary references do not suggest the elements missing from Bonnefoy and Lisowsky. Accordingly, this rejection cannot be sustained.

Rejection—35 U.S.C. §103(a)

Claim 6 was rejected under 35 U.S.C. §103(a) as being unpatentable over Bonnefoy, et al., Meth. Enzymol. 350: 97, in view of Lisowsky, et al., Eur. J. Biochem. 164:559, and further in view of Anziano, et al., PNAS 88:5592. Anziano was cited as teaching the COXII gene as a reporter, but does not remedy the deficiencies of Bonnefoy or Lisowsky as discussed above. Therefore, this rejection cannot be sustained.

Rejection—35 U.S.C. §103(a)

Claims 9 and 10 were rejected under 35 U.S.C. §103(a) as being unpatentable over Bonnefoy, et al., Meth. Enzymol. 350: 97, in view of Lisowsky, et al., Eur. J. Biochem. 164:559, and further in view of Fincham, Micro. Rev. 53:148. Fincham was cited as teaching cotransformation in yeast as required by claims 9 and 10. However, it does not teach the elements missing from Bonnefoy and Lisowsky. Therefore, this rejection cannot be sustained for the reasons discussed above.

Rejection—35 U.S.C. §103(a)

Claim 13 was rejected under 35 U.S.C. §103(a) as being unpatentable over Bonnefoy, et al., Meth. Enzymol. 350: 97, in view of Lisowsky, et al., Eur. J. Biochem. 164:559, and further in view of Kim, et al., Canc. Res. 57:3115. Kim was relied upon for teaching the lysing and centrifuging steps of claim 13. However, it does not teach the elements missing from Bonnefoy and Lisowsky. Accordingly, this rejection cannot be sustained for the reasons discussed above.

Rejection—35 U.S.C. §103(a)

Claim 14 was rejected under 35 U.S.C. §103(a) as being unpatentable over Bonnefoy, et al., Meth. Enzymol. 350: 97, in view of Lisowsky, et al., Eur. J. Biochem. 164:559, and further in view of Dziembowski, et al., J.B.C. 278:1603. and diRago, et al., J.B.C. 263:12564. Dziembowski has been addressed above, it in combination with diRago were also relied upon for disclosing particular buffers for lysing cells and organelles of claim 14. However, these references do not teach the elements missing from Bonnefoy and Lisowsky, namely expression of heterologous RNA using "yeast cells lacking mitochondrial DNA". Accordingly, this rejection cannot be sustained.

Conclusion

In view of the amendments and remarks above, the Applicants respectfully submit that this application is now in condition for allowance. An early notice to that effect is earnestly solicited.

Respectfully submitted,

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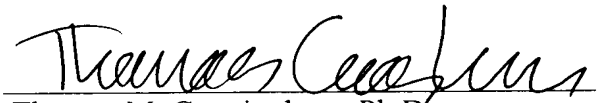

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